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Impact of Single Nucleotide Polymorphisms in Leptin, Leptin Receptor, Growth Hormone Receptor, and Diacylglycerol Acyltransferase (DGAT1) Gene Loci on Milk Production, Feed, and Body Energy Traits of UK Dairy Cows

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ABSTRACT

The impact of 9 single nucleotide polymorphisms (SNP) in the leptin (LEP), leptin receptor (LEPR), growth hormone receptor (GHR), and diacylglycerol acyltransferase (DGAT1) gene loci on daily milk production, feed intake, and feed conversion, and weekly measures of live weight, BCS, and body energy traits was evaluated using genetic and phenotypic data on 571 Holstein cows raised at the Langhill Dairy Cattle Research Center in Scotland. Six SNP were typed on the LEP gene and 1 on each of the other 3 loci. Of the 6 LEP SNP, 3 were in very high linkage disequilibrium, meaning there is little gain in typing all of them in the future. Seven LEP haplotypes were identified by parsimony-based analyses. Random-regression allele-substitution models were used to assess the impact of each SNP allele or haplotype on the traits of interest. Diacylglycerol acyltransferase had a significant effect on milk yield, whereas GHR significantly affected feed intake, feed conversion, and body energy traits. There was also evidence of dominance in allelic effects on milk yield and BCS. The LEP haplotype CCGTTT (corresponding to leptin SNP C207T, C528T, A1457G, C963T, A252T, and C305T, respectively) significantly affected milk yield and feed and dry matter intake. Animals carrying this haplotype produced 3.13 kg more milk daily and consumed 4.64 kg more feed. Furthermore, they tended to preserve more energy than average. Such results may be used to facilitate genetic selection in animal breeding programs.

Key words: leptin, growth hormone, diacylglycerol acyltransferase (DGAT1), milk

INTRODUCTION

Genomic data have the potential to contribute valuable information for animal selection and are being increasingly used in the genetic evaluation of animals and design of genetic improvement programs. Individual genes, markers or QTL can be considered to select desirable genotypes early in the life of a candidate parent. In this respect, knowledge of the association between individual genes and animal performance becomes essential for their effective use.

Several genes localized within QTL are thought to influence important traits in dairy cattle. Single nucleotide polymorphisms (**SNP**) are mostly used to genetically characterize such chromosomal regions. Leptin (**LEP**), leptin receptor (**LEPR**), growth hormone receptor (**GHR**), and diacylglycerol acyltransferase (**DGAT1**) are some pertinent examples.

The LEP gene, located on *Bos taurus* autosome (**BTA**) 4, encodes leptin, a protein hormone secreted from white adipose tissue that is involved in feed intake, energy partitioning, and metabolism of the cow (Liefers et al., 2002; Lagonigro et al., 2003). Consequently, the gene may also affect traits such as milk production, energy balance, and reproduction (Buchanan et al., 2002; Liefers et al., 2002; Silva et al., 2002). Furthermore, variation in leptin concentration during a cow's pregnancy is affected by polymorphisms in the LEPR gene (Liefers et al., 2004), located on chromosome BTA3, suggesting possible associations of the latter with cow traits linked to leptin.

Polymorphisms in the GHR gene, found on chromosome BTA20, have also been linked to milk production traits (Falaki et al., 1996; Blott et al., 2003). This gene controls the function of the receptor of the growth hormone, whose key role in mammary gland development and milk production has been well established (Bauman et al., 1985). The impact of the gene on other eco-

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Table 1. Descriptive statistics of milk production, feed, and body energy traits

Trait[1]	Unit of measure	Recording frequency	Records, n	Lactations, n	Mean	SD
MY	Kilogram	Daily	95,249	3	28.01	8.06
FI	Kilogram	Daily	95,249	3	51.51	12.61
DMI	Kilogram	Daily	95,249	3	17.38	4.39
FMR	Ratio	Daily	95,249	3	1.97	0.71
DMMR	Ratio	Daily	95,249	3	0.68	0.22
LW	Kilogram	Weekly	11,209	1	555.09	51.62
BCS	Scale 0–5	Weekly	11,209	1	2.69	0.32
EC	Megajoule	Weekly	11,209	1	4,786.98	699.08
CEEB	Megajoule	Weekly	11,209	1	142.07	933.37

[1]MY = milk yield; FI = feed intake; FMR = feed intake over milk yield ratio; DMMR = DMI over milk yield ratio; LW = live weight; BCS = BCS; EC = energy content; CEEB = cumulative effective energy balance.

nomically important traits in dairy cattle has not been fully investigated.

The DGAT1 gene, which is located on the BTA14 chromosomal site and encodes acyl coenzyme A:diacylglycerol acyltransferase, is known to catalyze the last step in triglyceride synthesis (Grisart et al., 2002) and is associated with milk yield and composition (Sanders et al., 2006; Gautier et al., 2007; Szyda and Komisarek, 2007). Its association with other important cow traits merits further attention.

The objective of this study was to determine if polymorphisms in the LEP, LEPR, GHR, and DGAT1 gene loci can provide useful genetic markers for daily milk yield, feed intake, and feed conversion as well as live weight, BCS, and body energy traits in UK Holstein cows.

MATERIALS AND METHODS

Data

Data involved 571 Holstein cows raised at the Langhill Dairy Cattle Research Centre in Scotland. These cows had calved between 1992 and 2002, and participated in a feed and selection trial. Thus, cows had been divided into control and selection lines, and sired by average and high genetic merit bulls, respectively. Furthermore, each line had been split into distinct groups that were fed high and low concentrate diets but, otherwise, were managed together.

Blood samples were drawn from these cows to determine their genotypes on the LEP, LEPR, GHR, and DGAT1 gene loci, as described below. Cow genotypes were then matched to individual cow production files. Daily phenotypic records were collected on the farm for milk yield, feed intake, and dry matter intake. These records were available for the first 3 lactations. Measures of feed conversion to milk yield were calculated as the ratios of feed and dry matter intake over milk yield. Furthermore, weekly first-lactation live weight and BCS records were obtained. These traits were combined to produce estimates of total body energy content and cumulative effective energy balance. The first of these energy traits is a measure of the actual energy level of the cow on the day of recording and the second a measure of the change in energy status as it accumulates throughout lactation. Detailed description of these traits can be found in Banos et al. (2006). Descriptive statistics of all traits considered in the present study are shown in Table 1.

Genetic Analysis

Cow genotypes were determined at the 4 gene loci. Within the LEP gene locus, genotypes were determined based on the following 6 SNP: C207T and C528T (Nkrumah et al., 2005; also referred to as UASMS-1 and UASMS-2, respectively) and A1457G and C963T (Liefers et al., 2005) located at the LEP promoter region, and A252T (Lagonigro et al., 2003; also referred to as E2JW) and C305T (Buchanan et al., 2002; Lagonigro et al., 2003; also referred to as EXON-2-FB or E2FB) found at the LEP exon 2 region. The nomenclature shown here demonstrates the substitution of one base for the other. For example, C305T refers to a cytosine to thymine base change in the LEP exon 2 region on BTA4. Furthermore, the number in each SNP name corresponds to a position sequence relevant to the appropriate accession number, as originally published. The SNP C207T and C528T are located on positions 207 and 528, respectively, of the LEP promoter region according to GenBank accession no. AB070368. The numbers in SNP A1457G and C963T refer to the distance from the onset of the transcription site (position 1600) of exon 1 as per GenBank Accession No. AJ571871; thus, the actual positions of SNP A1457G and C963T are 143 (1600 – 1457) and 637 (1600 – 963), respectively, on the LEP promoter region. The correspondence of these 2 GenBank accession numbers is such that AJ571871 begins at position 1398 of

Table 2. Genotype and allele frequencies (%) in the 4 gene loci

		Allele[1]		Genotype frequency (%)			Allele frequency[2] (%)	
Gene locus	SNP	0	+	00	0+	++	0	+
Leptin	C207T	C	T	13	46	42	36	64
	C528T	C	T	82	17	1	90	10
	A1457G	A	G	27	51	22	52	48
	C963T	C	T	42	45	13	65	35
	A252T	A	T	95	5	0	97	3
	C305T	C	T	43	45	12	65	35
Leptin receptor	T945M	C	T	86	14	1	93	7
Growth hormone receptor	F279Y	T	A	63	34	3	80	20
DGAT1[3]	K232A	Q	P	25	45	30	47	53

[1]T = thymine, C = cytosine, A = adenine, G = guanine, Q = variant coding for lysine, P = variant coding for alanine.

[2]Approximate standard errors 0.7 to 2.4%.

[3]DGAT1 = diacylglycerol acyltransferase.

AB070368. The numbers on the LEP exon 2 region SNP A252T and C305T refer to positions 252 and 305, respectively, as per the EMBL accession no. AY138588.

One additional SNP was determined at each of the LEPR (Liefers et al., 2004), GHR (Blott et al., 2003), and DGAT1 (Grisart et al., 2002) gene loci. The SNP at the LEPR and GHR loci characterize a cytosine to thymine and a thymine to adenine base substitution, respectively, whereas a lysine to alanine amino acid change occurs at the DGAT1 locus.

All genetic analyses were conducted by Igenity (Igenity is a registered trademark of Merial in the United States and elsewhere). Briefly, blood samples were retained from all cows that contributed to the database, and DNA was extracted following a phenol-chloroform based protocol. The genotyping process used was allele-specific primer extension reactions analyzed with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Table 2 summarizes the 9 SNP in the 4 loci.

Once genotypes were determined, allele frequencies at each SNP locus were calculated by simple gene counting. This approach may be slightly biased by assumptions of random mating and no selection. These assumptions were tested for individual loci by examining deviations from Hardy-Weinberg equilibrium using chi-squared tests. Approximate standard errors of allele frequencies were calculated by the square root of $P(1 - P)/n$, where P = allelic frequency and n = number of cows), which decreases as the minimum allelic frequency tends toward zero.

A minimal set of haplotypes comprising the 6 SNP in the LEP gene locus were determined using parsimony, both by genotype observation and custom software. Parsimony, as described by Clark (1990), is based on the assignment of haplotypes to genotypes that minimizes the number of unassigned or unresolved haplotypes (termed orphans by Clark, 1990). In practice, definite and unambiguous haplotypes are identified first from homozygous individuals and then from individuals with only 1 heterozygous SNP locus (Tier, 2006). Following that, unique pairs of haplotypes are assigned, where possible, to their genotypes. In the present study, data were restricted to 491 cows with all 6 LEP SNP available. Given the animal genotype for these 6 SNP, an algorithm was developed to test every plausible pair of haplotypes against the genotypes. A putative pair of haplotypes that corresponded to the LEP genotype was assigned to each of these animals.

Linkage disequilibrium among the 6 SNP loci was derived from the haplotypes and was calculated as $D(i,j) = P(i,j) - P(i)P(j)$, for specific alleles at SNP loci i and j, using the haplotype $[P(i,j)]$ and allele $[P(i), P(j)]$ frequencies for each SNP locus inferred from them. The D coefficients were then normalized by dividing them by the theoretical maximum for the observed allelic frequencies (Lewontin, 1964), where the latter was the smaller of $P(i)[1 - P(j)]$ and $[1 - P(i)]P(j)$ for $D \geq 0$ or the larger of $-P(i)P(j)$ and $-[1 - P(i)][1 - P(j)]$ for $D < 0$. The D coefficients were also normalized by dividing them by the geometric mean of the binomial variances to obtain the final disequilibrium values. In the case of 2 biallelic loci, the latter is equivalent to a correlation coefficient defined by the quotient between D and the square root of the product of the 4 allelic frequencies, as shown in equation 1:

$$r = \frac{D}{\sqrt{Pi \cdot (1 - Pi) \cdot Pj \cdot (1 - Pj}}, \qquad [1]$$

where r = final value of linkage disequilibrium (Hill and Robertson, 1968). As this correlation approaches unity, the second locus becomes redundant.

Statistical Analysis

Daily milk production and feed trait records (feed and dry matter intake and their ratios over milk yield) were analyzed with the following random regression model (model 2):

$$Y = m + ys + g + f + lc + ph + age + GEN + \Sigma_k b_k P_k(d) + \Sigma_k a_k P_k(d) + e, \quad [2]$$

where Y = daily milk production or feed trait record; m = overall mean; ys = fixed effect of year-by-season of calving interaction (35 classes); g = fixed effect of genetic line (2 classes); f = fixed effect of diet group (2 classes); lc = fixed effect of lactation number (3 classes); ph = linear regression on percentage of Holstein (North American) genes; age = linear regression on age at calving; GEN = regression on individual SNP alleles or haplotypes expressed as 0, 1, or 2 as described below; $\Sigma_k b_k P_k(d)$ = fixed regression of the overall lactation curve (b_k) described by fourth-degree Legendre polynomials (P_k) on DIM (d); $\Sigma_k a_k P_k(d)$ = random regression of the individual cow effect (a_k) described by third-degree Legendre polynomials (P_k) on DIM (d) (the term included pedigree genetic relationships among animals); and e = random residual.

In model 2, the SNP allelic effect was described as 0, 1, or 2, in each case corresponding to as many copies of the substitution SNP base. For example, LEP SNP C207T includes the bases cytosine and thymine. For this SNP, the GEN variable in model 2 would be assigned values 0, 1, and 2 for cows with TT, CT, and CC genotypes, respectively. This yields the effect of base C, equivalent to the allele substitution effect at this SNP locus at the observed frequency. Thus, model 2 is an allele substitution model.

The analysis was repeated for each SNP locus and trait. In a separate series of analyses, interactions between alleles in a SNP locus were also fitted to assess possible dominance effects. In yet another set of analyses, LEP haplotypes were fitted in the model, each one separately, instead of individual LEP SNP.

In model 2, the random regression corresponded to the polygenic effect of each cow. All known pedigree information was included, bringing the total number of animals in the analysis to 968.

Live weight, BCS, energy content, and cumulative effective energy balance, which henceforth will be collectively referred to as body energy traits, were analyzed with a similar model, except that lactation number was omitted (because there were only first-lactation records) and milk yield on the day of measurement was included as a covariate, to account for the phenotypic correlation between milk production and body energy. The trajectory in these analyses was defined by week of lactation. Otherwise, the exact same analyses were conducted as for milk and feed traits. Variance components and fixed effect solutions were calculated using the ASREML software package (Gilmour et al., 2002).

In each of the above analyses, the null hypothesis was of no SNP or haplotype effect on the trait of interest. Inevitably, multiple hypothesis tests were made because several traits were analyzed. However, these traits are genetically correlated to each other, so not all analyses constitute truly independent statistical tests. To account for multiple testing, we considered the analyses of (i) milk yield and feed and dry matter intake, (ii) feed conversion, and (iii) body traits as distinct, independent hypothesis tests. A Bonferroni correction, based on the Holm-Bonferroni method (Holm, 1979), was then implemented to separately test each hypothesis. This method uses the ratio of the original significance level (e.g., 0.05) over the number of hypothesis tests as the new significance threshold. The method works sequentially starting with the test with the lowest unadjusted *P*-value, which it compares with 0.05/k, where k is the number of hypotheses tests. If this hypothesis is rejected, the next unadjusted *P*-value is compared with 0.05/(k – 1) and so on, until a hypothesis is not rejected (Holm, 1979).

RESULTS

Genetic Structure

Table 2 shows genotype and allele frequencies estimated for the 4 gene loci. Within the LEP gene, separate estimates were derived for the 6 individual SNP loci. The maximum estimated standard error for all loci was less than 2.5%.

Grading-up selection favoring certain alleles and the finite number of parents (in populations undergoing sexual reproduction) may lead to departures from Hardy-Weinberg equilibrium, that is, the classical genotypic frequencies of p^2, $2p(1 - p)$ and $(1 - p)^2$, where p is the frequency of 1 of the 2 alleles. The only locus showing a statistically significant ($P < 0.05$) departure from equilibrium was the DGAT1 gene locus, with a deficit of heterozygotes in favor of both homozygotes of approximately 10% from the expected value. In contrast, the polymorphism in the GHR gene locus displayed an excess of heterozygotes, of approximately 9% over expectations, although this deviation was only suggestive, because it did not attain statistical significance ($P > 0.05$). These departures are relatively small and are difficult to interpret without further data and more detailed analysis fully accounting for the population structure. The estimates of allele frequencies given in Table 2 assume random mating.

Table 3. Leptin gene haplotypes and number of copies per haplotype, calculated for the 490 cows with all leptin single nucleotide polymorphisms (SNP) identified

Haplotype[1]	Number of copies inferred	Frequency, %
CCGTAT	328	33.4
TCACAC	402	41.0
TCGCAC	124	12.7
TTACAC	96	9.8
CCACAC	1	0.1
CCGTTT	26	2.7
CCGTAC	3	0.3
Total	980	100

[1]SNP order: C207T, C528T, A1457G, C963T, A252T, C305T.

Eight LEP haplotypes were identified by parsimony. The order of the 6 corresponding LEP SNP was C207T, C528T, A1457G, C963T, A252T, and C305T, according to their physical position on the gene; the first 4 SNP are located in the promoter and the last 2 in the exon 2 region. Table 3 shows the number of copies for each of 7 of the haplotypes. In total, the number of copies is twice the number of animals (490 cows with all LEP SNP identified) because each cow bears 2 haplotype copies. The remaining haplotype pertained to 1 animal and could not be precisely defined but was of the form TCXCAT, where X is most likely to be A. This cow was excluded from the analysis. For all other animals, the allocation of pairs of haplotypes was uniquely defined by the observed genotypes and the algorithm developed for this purpose.

Absolute linkage disequilibrium values calculated for the 6 LEP SNP loci and normalized by their theoretical maxima ranged from 0.995 to 1. It should be noted that the use of parsimony to determine linkage disequilibrium measures is likely to inflate the magnitude of the disequilibrium. Therefore, these values are considered to be at the maximum magnitude. The interpretation of such values is that no recombination has taken place between these loci since the latest mutation occurred. This is perhaps unlikely in the population of dairy cattle as a whole; however, it is indicative of a high degree of linkage. Furthermore, it supports the no-recombination assumption that is implicit to the haplotype reconstruction procedure.

Linkage disequilibrium values normalized by the geometric mean of the binomial variances ranged from –0.222 to 0.998. Such values approach a magnitude of 1 when the occurrence of the allele at an SNP locus perfectly predicts the allele at the other SNP locus. In this data set, large (>0.99) disequilibrium values occurred among 3 SNP loci: C207T, C963T, and C305T. This means that little is to be gained in genotyping more than 1 SNP from this set of loci. Furthermore, linkage disequilibrium values between these 3 SNP loci and SNP A1457G ranged from 0.765 to 0.770, suggesting that variation in the latter may add some additional information, but is also relatively highly correlated with variation at the first set of 3 SNP loci. In all other cases, linkage disequilibrium values were smaller, supporting the need to genotype the SNP to improve characterization of the LEP gene locus.

Milk and Feed Traits

Table 4 shows the genetic effects on milk production and feed traits when each SNP was fitted individually into the model. These effects refer to substituting the allele shown in Table 4 for the other allele at the corresponding SNP locus. For example, according to Table 4, substituting one copy of base (allele) A for a copy of base T at the A252T LEP SNP was associated with a significant ($P < 0.05$) reduction of daily milk yield by 2.3 kg. Similarly, replacing a copy of base A by a copy of base T at the GHR SNP locus decreases the daily milk yield by 0.52 kg, although this effect was not significantly different from zero ($P > 0.05$).

In general, significant ($P < 0.05$) allelic substitution effects were found for LEP SNP A252T on milk yield and A1457G on milk yield and dry matter intake, the GHR SNP on feed and dry matter intake and feed to milk ratios, and the DGAT1 SNP on milk yield (Table 4). Following the Bonferroni correction, however, only the effect of the DGAT1 SNP on milk yield and the GHR SNP effects remained significant. A few other effects with preadjusted significance levels between 0.05 and 0.10 were also found, suggesting possible but not statistically established associations (Table 4).

When dominance was fitted in the model, LEP SNP A1457G had a significant ($P < 0.05$, even after the Bonferroni correction) effect of -0.88 ± 0.37 kg on daily milk yield. The estimate of the additive effect in this case was -0.65 ± 0.32 kg ($P < 0.05$, but not significant following Bonferroni correction) and represents half the difference between the homozygotes, whereas the dominance coefficient is the deviation from this estimate (Falconer, 1983). Results suggest nearly complete dominance of base A over G at this SNP locus, meaning that milk yield of animals with the AG genotype is much more similar to milk yield of AA animals than to that of GG animals. The estimate of the additive effect when dominance was included was very similar to the estimate from the additive-only model (Table 4). This is probably the result of allelic frequencies being close to 0.50 in the SNP locus A1457G (Table 2), meaning that the allelic substitution effect is independent of allele frequency. With this dominance relationship, the magnitude of the allelic substitution effect will increase (or decrease) as G increases (or decreases) in frequency.

Table 4. Allele substitution effect (α) on milk production and feed traits

Trait	Gene - SNP	Substitution allele	α (SE)	*P*-value
Milk yield (kg/d)	Leptin - C207T	C	0.56 (0.33)	0.09
	Leptin - C528T	C	0.62 (0.56)	0.26
	Leptin - A1457G	A	–0.70 (0.33)	0.03[a]
	Leptin - C963T	C	–0.53 (0.33)	0.11
	Leptin - A252T	A	–2.30 (1.05)	0.03[a]
	Leptin - C305T	C	–0.46 (0.33)	0.17
	Leptin receptor	C	0.03 (0.66)	0.97
	Growth hormone receptor	T	–0.52 (0.40)	0.19
	DGAT1[1]	Q	–0.79 (0.34)	0.02[a,b]
Feed intake (kg/d)	Leptin - C207T	C	0.79 (0.55)	0.15
	Leptin - C528T	C	0.46 (0.94)	0.62
	Leptin - A1457G	A	–0.90 (0.55)	0.10
	Leptin - C963T	C	–0.83 (0.55)	0.13
	Leptin - A252T	A	–2.33 (1.76)	0.19
	Leptin - C305T	C	–0.86 (0.56)	0.12
	Leptin receptor	C	–0.17 (1.10)	0.88
	Growth hormone receptor	T	1.72 (0.67)	0.01[a,b]
	DGAT1	Q	0.60 (0.52)	0.25
DMI (kg/d)	Leptin - C207T	C	0.23 (0.14)	0.11
	Leptin - C528T	C	0.14 (0.24)	0.58
	Leptin - A1457G	A	–0.32 (0.14)	0.03[a]
	Leptin - C963T	C	–0.24 (0.14)	0.10
	Leptin - A252T	A	–0.85 (0.46)	0.06
	Leptin - C305T	C	–0.24 (0.15)	0.09
	Leptin receptor	C	–0.12 (0.29)	0.67
	Growth hormone receptor	T	0.42 (0.18)	0.02[a,b]
	DGAT1	Q	0.12 (0.14)	0.37
Feed intake over milk yield	Leptin - C207T	C	–0.03 (0.03)	0.31
	Leptin - C528T	C	–0.02 (0.06)	0.70
	Leptin - A1457G	A	0.05 (0.03)	0.13
	Leptin - C963T	C	0.03 (0.03)	0.37
	Leptin - A252T	A	0.13 (0.11)	0.24
	Leptin - C305T	C	0.02 (0.03)	0.50
	Leptin receptor	C	–0.01 (0.07)	0.93
	Growth hormone receptor	T	0.14 (0.04)	0.00[a,b]
	DGAT1	Q	0.05 (0.03)	0.08
DMI over milk yield	Leptin - C207T	C	–0.02 (0.01)	0.15
	Leptin - C528T	C	–0.02 (0.02)	0.19
	Leptin - A1457G	A	0.02 (0.01)	0.13
	Leptin - C963T	C	0.01 (0.01)	0.18
	Leptin - A252T	A	0.05 (0.03)	0.15
	Leptin - C305T	C	0.01 (0.01)	0.25
	Leptin receptor	C	0.00 (0.02)	0.93
	Growth hormone receptor	T	0.04 (0.01)	0.00[a,b]
	DGAT1	Q	0.02 (0.01)	0.07

[a]α values are significant at unadjusted $P < 0.05$; [b]α values are significant after a Bonferroni correction.
[1]DGAT1 = diacylglycerol acyltransferase.

Dominance effects at other LEP SNP loci were not different from zero ($P > 0.05$).

The GHR effects on feed and dry matter intake also showed significant ($P < 0.05$) dominance, with estimated effects of 1.86 ± 0.89 and 0.61 ± 0.23 kg, respectively. However, this result was not significant after the Bonferroni correction. Corresponding allele substitution (additive) effects for base T were 2.85 ± 0.86 kg ($P < 0.05$, significant after Bonferroni correction) and 0.79 ± 0.22 kg ($P < 0.05$, significant after Bonferroni correction), slightly greater than those from the additive-only model (Table 4). These values suggest the T allele may be partially dominant, such that cows with AT genotypes consume feed at a rate more similar to cows with TT than with AA. Because T is the most common allele (frequency of 0.80; Table 2), the partial dominance reduces the effect of its substitution on selection and breeding.

When an additive-only model is fitted, the estimate obtained is the effect of the allele substitution on breeding value. In such a case, if there is any dominance present, the additive estimate will vary with the gene frequency. As explained earlier, when dominance is fitted explicitly, the additive regression coefficient represents an estimate of half the difference between the homozygotes and the dominance coefficient is the devia-

Table 5. Leptin gene haplotype substitution effect (h) on milk production and feed traits

Trait	Haplotype	h (SE)	P-value
Milk yield (kg/d)	CCGTAT	0.13 (0.33)	0.71
	TCACAC	–0.42 (0.34)	0.21
	TCGCAC	0.38 (0.48)	0.43
	TTACAC	–0.46 (0.57)	0.42
	CCGTTT	3.13 (1.16)	0.01[a,b]
Feed intake (kg/d)	CCGTAT	0.46 (0.59)	0.43
	TCACAC	–0.94 (0.60)	0.12
	TCGCAC	0.24 (0.85)	0.78
	TTACAC	–0.13 (1.01)	0.90
	CCGTTT	4.64 (2.05)	0.02[a,b]
DMI (kg/d)	CCGTAT	0.13 (0.15)	0.40
	TCACAC	–0.31 (0.16)	0.04[a]
	TCGCAC	0.23 (0.22)	0.30
	TTACAC	–0.12 (0.26)	0.66
	CCGTTT	1.29 (0.54)	0.02[a,b]
Feed intake over milk yield	CCGTAT	–0.01 (0.03)	0.77
	TCACAC	0.03 (0.04)	0.43
	TCGCAC	–0.04 (0.05)	0.37
	TTACAC	0.03 (0.06)	0.59
	CCGTTT	–0.08 (0.12)	0.51
DMI over milk yield	CCGTAT	–0.01 (0.01)	0.55
	TCACAC	0.00 (0.01)	0.72
	TCGCAC	0.00 (0.02)	0.84
	TTACAC	0.02 (0.02)	0.19
	CCGTTT	–0.05 (0.04)	0.21

[a]h values are significant at unadjusted $P < 0.05$; [b]h values are significant after a Bonferroni correction.

tion. The additive part assumes additivity given the absence of both heterozygotes. The dominance deviation contributes to the offspring only to a certain degree, which depends on the allele frequency. Therefore, the additive effects from an additive-only model are the appropriate estimates when considering the effect of these loci on selection and breeding.

All previous results pertained to fitting each SNP separately in the model. Fitting all LEP SNP simultaneously would yield marginal effect estimates but also encounter difficulties given the high degree of linkage disequilibrium among the individual SNP loci. In light of this observation, more reliable results may be obtained from estimating substitution effects of inferred LEP gene haplotypes. This allows for some aspects of epistasis among the loci and works with causes of disequilibrium rather than trying to treat them as independent.

Estimates of haplotype substitution effects on milk and feed traits are shown in Table 5. The CCGTTT haplotype had a significant ($P < 0.05$) positive impact on milk yield and feed and dry matter intake, which remained significant after the Bonferroni correction. One copy of the haplotype is associated with an additional 3.13 kg of milk being produced and 4.64 and 1.29 kg more feed and dry matter, respectively, being consumed by the cow daily. This haplotype also had the largest magnitude of effect on the 2 feed to milk ratios (in fact reducing the ratio), but estimates were not significantly different from zero. The outcome is consistent with the allele substitution analyses, because it contains the G allele for SNP A1457G and it is also the only haplotype with the T allele for SNP A252T. The TCACAC haplotype was the only other LEP haplotype that seemed associated with a trait, namely daily dry matter intake (–0.31 ± 0.16 kg), but this effect was not significant post-Bonferroni correction.

Body Energy Traits

Both the structure and comments concerning fitting the different models are similar to the previous section on milk production and feed traits. Table 6 shows the allele substitution effects on body energy traits when loci were fitted singly into models including additive allelic effects. The smaller amount of data resulted in most effects being nonsignificant. Only the GHR gene had a significant ($P < 0.05$) effect on cumulative effective energy balance, with the T allele being associated with reduced body energy. This effect was significant even after the Bonferroni correction. In addition, the DGAT1 locus was found to have a marginal effect on cumulative effective energy balance ($P = 0.05$), with the variant coding for lysine being associated with more energy accumulated. Furthermore, LEP SNP A252T suggested a potential effect ($P = 0.08$) on BCS, where the A allele may be indicative of compromised body condition (Table

Table 6. Allele substitution effect (α) on body energy traits

Trait	Gene - SNP	Substitution allele	α (SE)	*P*-value
Live weight (kg)	Leptin - C207T	C	1.62 (3.39)	0.63
	Leptin - C528T	C	–3.17 (6.49)	0.63
	Leptin - A1457G	A	0.56 (3.24)	0.86
	Leptin - C963T	C	–1.62 (3.39)	0.63
	Leptin - A252T	A	–11.89 (10.11)	0.24
	Leptin - C305T	C	–2.51 (3.44)	0.47
	Leptin receptor	C	0.41 (7.40)	0.96
	Growth hormone receptor	T	3.80 (4.54)	0.40
	DGAT1[1]	Q	0.68 (3.59)	0.85
BCS (scale 0–5)	Leptin - C207T	C	0.01 (0.02)	0.76
	Leptin - C528T	C	–0.04 (0.04)	0.29
	Leptin - A1457G	A	0.00 (0.02)	0.82
	Leptin - C963T	C	–0.01 (0.02)	0.76
	Leptin - A252T	A	–0.10 (0.06)	0.08
	Leptin - C305T	C	–0.01 (0.02)	0.65
	Leptin receptor	C	0.05 (0.04)	0.26
	Growth hormone receptor	T	0.00 (0.02)	0.84
	DGAT1	Q	–0.02 (0.02)	0.37
Energy content (MJ)	Leptin - C207T	C	17.29 (46.62)	0.71
	Leptin - C528T	C	–75.40 (88.77)	0.40
	Leptin - A1457G	A	8.43 (44.40)	0.85
	Leptin - C963T	C	–17.29 (46.62)	0.71
	Leptin - A252T	A	–216.8 (139.7)	0.12
	Leptin - C305T	C	–27.97 (47.30)	0.55
	Leptin receptor	C	55.53 (100.8)	0.60
	Growth hormone receptor	T	32.14 (62.44)	0.61
	DGAT1	Q	–23.85 (48.33)	0.62
Cumulative effective energy balance (MJ)	Leptin - C207T	C	–11.76 (20.62)	0.57
	Leptin - C528T	C	26.34 (38.75)	0.50
	Leptin - A1457G	A	8.95 (19.95)	0.65
	Leptin - C963T	C	11.76 (20.62)	0.57
	Leptin - A252T	A	–28.54 (63.14)	0.65
	Leptin - C305T	C	14.87 (20.91)	0.48
	Leptin receptor	C	–2.21 (45.37)	0.96
	Growth hormone receptor	T	–87.30 (27.51)	0.00[a,b]
	DGAT1	Q	1.18 (20.65)	0.05

[a]α values are significant at unadjusted $P < 0.05$; [b]α values are significant after a Bonferroni correction.
[1]DGAT1 = diacylglycerol acyltransferase.

6). None of the SNP studied seemed to individually affect live weight and energy content.

The LEP SNP C528T and A1457G were associated with significant ($P < 0.05$) dominance effects on body condition that amounted to 0.19 ± 0.09 and 0.06 ± 0.02, respectively. However, only the latter was still significant after the Bonferroni correction. Additive effects in these SNP loci were nonsignificant ($P > 0.05$) after fitting a dominance interaction. In the first instance, these results are notable because they suggest over-dominance, which is not a common feature in well-documented loci. Admittedly, the estimate of the additive effect of the C528T locus (from a dominance model) was 0.13 ± 0.08, which, although nonsignificant ($P = 0.11$), may be indicative of complete dominance with C being dominant to T and associated with improved body condition. Given that C is the most common allele at this SNP locus (frequency of 0.9; Table 2), the estimate of the additive effect in the population is considered small in magnitude. The additive effect at A1457G was 0.00 ± 0.02 ($P = 0.82$) and appears to more clearly suggest over-dominance. Dominance effects on all other loci were statistically not different from zero ($P > 0.05$).

The estimates for LEP haplotypes are given in Table 7. Nonsignificant ($P > 0.05$) effects were observed, but there was a suggestion for the CCGTTT haplotype to be associated with somewhat heavier animals ($P = 0.10$) with more body condition ($P = 0.12$) and more energy content ($P = 0.08$).

DISCUSSION

This analysis of the studied genotypes suggests certain significant effects on milk yield, feed intake, feed conversion, and body energy traits based on allele and haplotype substitution effect models fitted to all available data plus pedigree information. The effect of an allele or haplotype substitution, as estimated here,

Table 7. Leptin gene haplotype substitution effect (h) on body energy traits

Trait	Haplotype	h (SE)	P-value
Live weight (kg)	CCGTAT	1.50 (3.38)	0.66
	TCACAC	–1.57 (3.41)	0.65
	TCGCAC	–5.31 (4.95)	0.28
	TTACAC	3.04 (6.62)	0.65
	CCGTTT	18.21 (10.95)	0.10
BCS (scale 0–5)	CCGTAT	0.00 (0.02)	0.93
	TCACAC	–0.01 (0.02)	0.78
	TCGCAC	–0.02 (0.03)	0.45
	TTACAC	0.03 (0.04)	0.44
	CCGTTT	0.09 (0.06)	0.12
Energy content (MJ)	CCGTAT	6.50 (46.45)	0.89
	TCACAC	–20.54 (46.69)	0.66
	TCGCAC	–59.49 (68.43)	0.38
	TTACAC	61.37 (90.33)	0.50
	CCGTTT	264.2 (151.4)	0.08
Cumulative effective energy balance (MJ)	CCGTAT	–6.92 (20.36)	0.73
	TCACAC	21.94 (20.60)	0.29
	TCGCAC	4.67 (30.92)	0.88
	TTACAC	–54.58 (38.77)	0.16
	CCGTTT	–17.85 (67.09)	0.79

measures the effect that substituting one variant for another has upon the individual animal's breeding value and expected performance.

For the DGAT1 and GHR genotypes, the effects on milk yield and its components have been well established during their discovery and validation, primarily by QTL detection in granddaughter designs (Grisart et al., 2002; Blott et al., 2003). The results from the present study are, in general, consistent with previous work in dairy cattle showing that the allele responsible for lysine is usually associated with decreased milk production (Spelman et al., 2002; Thaller et al., 2003; Szyda and Komisarek, 2007). These studies also reported increasing milk fat concentration in animals carrying the lysine variant, but milk components were not included in the present analysis.

At the GHR locus, the studied mutation involved a thymine to adenine base substitution. Results of the present study suggested a positive but not significant ($P = 0.19$) effect of adenine on daily milk production. Blott et al. (2003) reported favorable effects of the same allele on daughter milk yield deviations of Dutch and New Zealand bulls.

For the most part, the published studies have estimated, by virtue of their design, the effect of an allele substitution and have been less able to detect dominance among the alleles. The degree of dominance was estimable in the present study, but there was no statistically significant evidence for any departure from additivity at either locus for milk yield.

A further benefit of this study in relation to DGAT1 and GHR gene loci is the availability of data on daily feed intake and body energy, which is not routinely recorded in commercial populations. Therefore, little information has been available on the associations of these loci with such traits. In the present study, the DGAT1 allele responsible for reduced milk production appeared to have a marginal positive effect on cumulative effective energy balance, suggesting that carriers are more likely to conserve energy than expend it for milk production. Furthermore, we found a significant impact of the GHR gene locus on feed and dry matter intake, with the thymine base allele being associated with increased intake and feed to milk ratios and, consequently, poorer feed conversion. There was also evidence that the thymine base allele was partially dominant in these actions. The effect of allelic substitution at the GHR SNP locus on these traits will increase as the allele frequency for adenine, which is likely to be positively associated with milk production, increases. Cows carrying this base also accumulated more energy balance over a lactation (87.3 MJ/copy). This appears to be the case when efficient conversion of feed to milk does not necessitate mobilization of the animals' energy reserves, which accumulate as lactation progresses. This picture may reflect the potential impact of the allele on milk components, which may alter apparent benefits.

Polymorphism in LEPR did not affect significantly any of the traits studied here. Minimal association between this gene and milk production was reported by Szyda and Komisarek (2007) in a study of Polish Holstein cattle.

The impact of variation in the LEP gene locus on dairy production has not been established by classical genome mapping, and its study has mostly followed a candidate gene approach (Buchanan et al., 2002; Lagonigro et al., 2003). In such circumstances, mutations

with significant effects may not be causal but may instead reflect linkage disequilibrium with mutations in other genes potentially at some distance from the LEP gene. In this context, 2 LEP SNP (A252T and A1457G) appeared to be associated with increased daily milk yield by 2.3 kg (in favor of thymine) and 0.7 kg (in favor of guanine), respectively. From a practical point of view, it may be tempting to dismiss the thymine base allele and the associated haplotype as being rare (3%) and therefore not worthy of further pursuit. However, it is important to recognize that the allele with the greatest potential impact will frequently be rare with its value diminishing as it becomes more and more common in the population. Commercial cost-benefit realities will determine the utility of this result. In any case, the value of individual alleles to the population may be assessed by their average effect, which is measured by the product $(1 - p)\alpha$, where p is the allelic frequency and α the allele substitution effect; thus, for daily milk yield, the average effect of thymine in SNP locus A252T is $(1 - 0.03) \times 2.3 = 2.23$ kg, whereas the average effect of guanine in A1457G is $(1 - 0.48) \times 0.7 = 0.36$ kg.

The same LEP SNP genotypes that produced more milk were also found to consume proportionately more feed, confirming the results of Lagonigro et al. (2003). Because the increase in feed intake was proportional to that of milk yield, feed conversion remained unaffected. The effect of these genotypes on live weight and body energy traits was nonsignificant. In general, cows that do not lose body energy to support increases in milk yield may be ideal candidates for future bull mothers that will help rectify losses in cow body energy following increased yield at the population level.

Given the high degree of linkage disequilibrium identified among certain SNP at the LEP gene locus, the analysis using the haplotypes may be more reliable than individual SNP analyses. In this regard, LEP haplotype CCGTTT was identified as the one with the greatest substitution effect. This haplotype includes both favorable alleles (G and T for A1457G and A252T, respectively). The average effect of CCGTTT on daily milk yield, measured by the product $(1 - p)\alpha$, is estimated to be 3.05 kg. Although this haplotype was also associated with greater feed intake, the estimates of feed to milk ratios were slightly lower (albeit not significantly different from zero). Nevertheless, LEP haplotype CCGTTT was associated with somewhat heavier ($P = 0.10$), better-conditioned animals ($P = 0.12$) with increased total body energy content ($P = 0.08$). This association is consistent with greater feed consumption by animals bearing this haplotype. The relative economic weight of each of these traits will determine the overall economic importance of the haplotype.

Clearly, it would be interesting to further examine the effects of haplotype CCGTTT. If it is indeed favorable, it would be important to determine whether its low frequency (2.7% in our data) is due to a recent mutation or due to selection against these alleles for some unstudied trait.

CONCLUSIONS

Certain significant associations found in this study can support the development of breeding programs that use molecular information on the LEP, GHR, and DGAT1 gene loci for the genetic evaluation of animals for milk yield and body traits. Results were based on data from 571 cows, meaning that they should be validated with independent studies. The LEP gene was characterized by 6 SNP. Dense-genotyping a 20-cM region flanking the LEP gene would help separate out direct effects of the LEP gene from linked effects. Furthermore, examination of pleiotropic effects on other traits such as fertility should be pursued. Finally, the evaluation of the environmental impact of these alleles given their effects on feed and feed to product ratios is warranted.

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